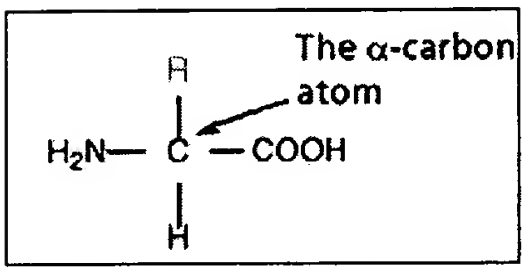
Proteins

2. The α-amino acid building blocks



2.1 The α-amino acids

Amino acids are the monomer building blocks from which proteins are made. In general an acid is an organic molecule that contains both a carboxylic acid (-COOH) and an amine ($-NH_2$) functional group. The acids that make proteins are all α-amino acids. In these acids both functional groups are attached to the same carbon atom (the α-carbon atom) at one end of the molecule. The simplest way to draw the structure of an α-amino acids is shown on the left.

In this diagram R stands for the rest of the molecule - the side chain group. R is the part of the molecule that makes one α-amino acid different from another. There are some twenty different α-amino acidsthat can make up proteins, so there are twenty different possibilities for the structure of the side chain group (see Figure 3).

2.2 Amino acids in 3D

The structures you have seen so far give us only a limited picture of what α-amino acid molecules may look like; they show the atoms that the molecules contain and how these atoms are linked together. They give no information about the 3-dimensional shape of the molecule - how the atoms are arranged in space. In some cases a pair of molecules that share the same sequence of atoms can have different arrangements in space called configurations. This leads to a type of isomerism called stereoisomerism.

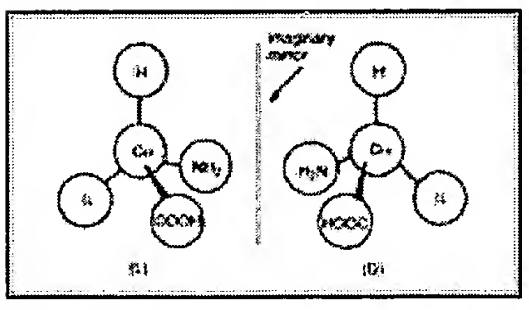


Figure 1
α-amino acids in 3D.

Almost all α-amino acids display a particular type of stereoisomerism that comes from the molecules being chiral . This means that the two arrangements of the molecules are non-identical mirror images, in the same way that your left hand is a non-identical mirror image of your right hand (see Figure 1). Non-identical mirror images are sometimes described as non-superimposable (you can't put a left hand glove on your right hand). The two molecules are called enantiomers.

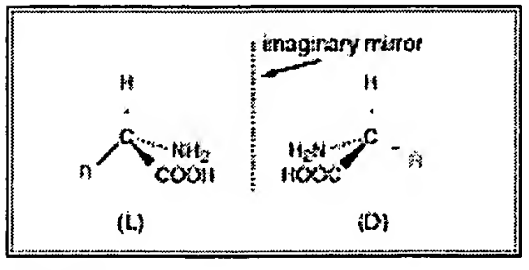
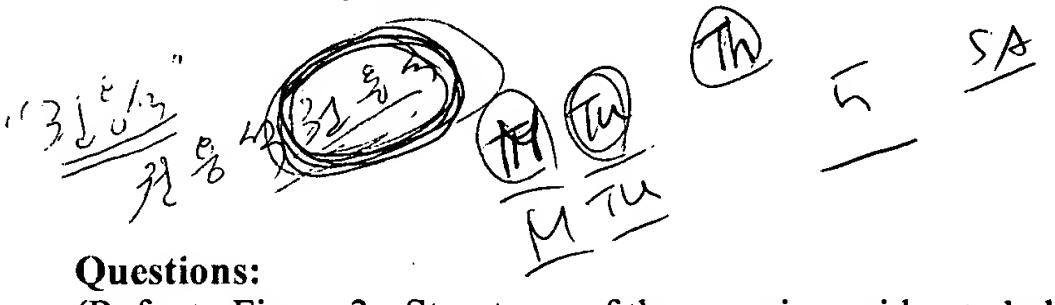
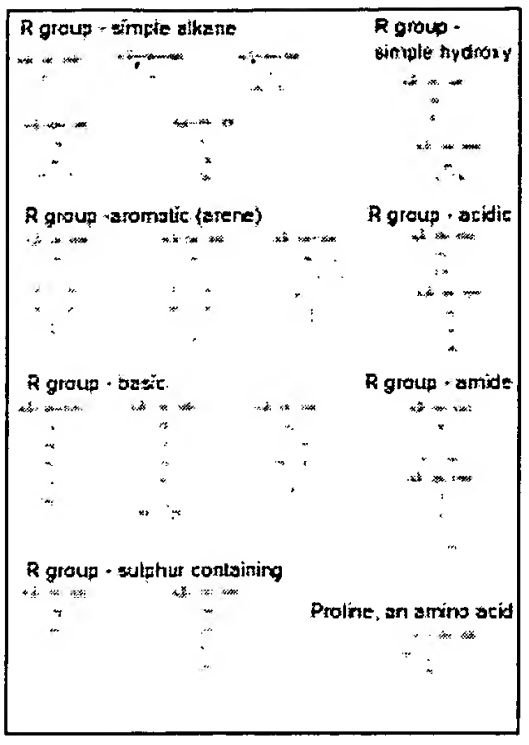


Figure 2
The conventional way to show the 3D shape of a molecule - in this case an amino acid.

Chirality in an α-amino acid is due to the α-carbon atom in the molecule being joined to four different atoms or groups. The α-carbon is called the chiral centre. We can draw the configurations of two enantiomers using a convention to show three dimensions (see Figure 2). Nearly all α-amino acids in nature share the same configuration, called the L configuration. Its mirror image is called the D configuration.



Questions:
(Refer to Figure 3 - Structures of the α–amino acids - to help you answer these questions.)



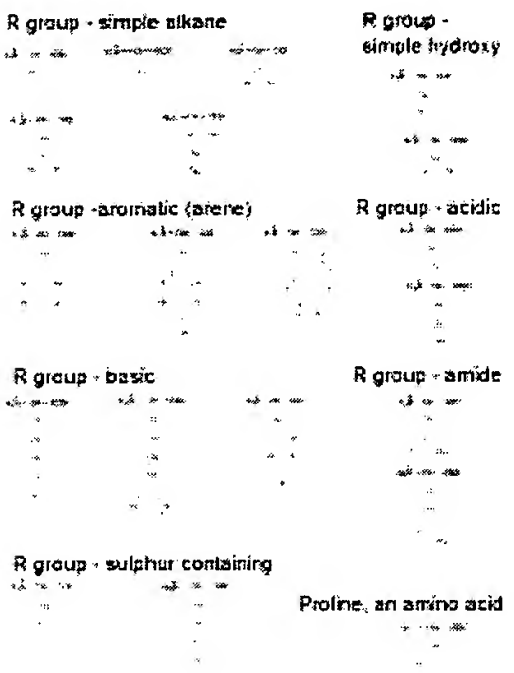
1. Glycine is the only α-amino acid that is not chiral . Explain why.

2. Which two α-amino acids have a chiral centre in their side group? Draw out the structures and label these centres.

3. Using 3D diagrams like those in Figure 2, draw out the configurations of L-alanine, D-alanine, L-cysteine and L-proline.

Figure 3. shows you the general shapes of the α-amino acids. When you cover a molecule with your mouse, its name will appear in the status bar at the bottom.
You can see a bigger diagram of a group of molecules by clicking on that group

Unilever **Proteins**



2. The α-amino acid building blocks

2.3 How can we group α-amino acids?

We can group α-amino acids acids by collecting together those with R groups that have similar features (Figure 3).

We can shorten the names of each α-amino acid using a one or three letter abbreviation. These abbreviations are given in Figure 3.

The side chain plays a large part in controlling the chemical and physical properties of an α-amino acid. Its solubility in water will depend on the polarity of the side chain group. A polar group will encourage solubility, a non-polar group will discourage it. For this reason α-amino acids are often divided into two groups, those with polar and those with non-polar side chain groups.

Figure 3. shows you the general shapes of the α-amino acids.
When you cover a molecule with your mouse, its name will appear in the status bar at the bottom.
You can see a bigger diagram of a group of molecules by clicking on that group

Questions:

1. Which α–amino acids have polar side chain groups?
2. Which α–amino acids have non-polar side chain groups?

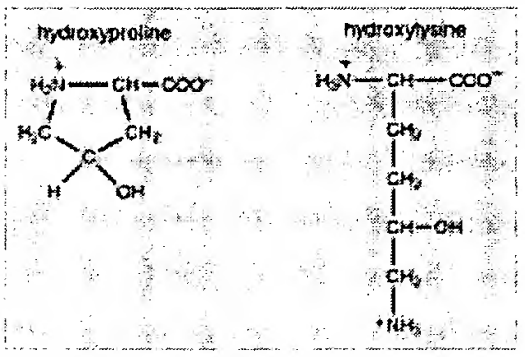


Figure 4
Two unusual α–amino acids.

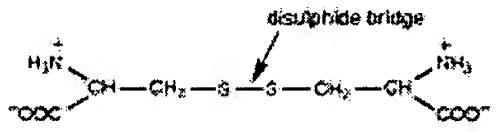


Figure 5
Cystine is a dimer of cysteine.

Proline is an unusual α–amino acid as the amino nitrogen is part of a secondary amino group (N-H) instead of the usual primary amino group (-NH2).

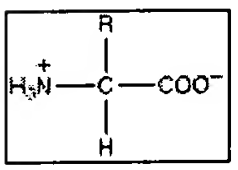
A few α–amino acids, not mentioned in Figure 3, occur in some proteins in a variety of organisms. The organism's biochemistry produces these by modifying the R groups of α–amino acids after they have been built into a protein. Proline can be modified to produce hydroxyproline and lysine can be modified to produce hydroxylysine (see Figure 4).

Cystine is an unusual α–amino acid in that it is a dimer formed by two cysteine side chain groups joining together (see Figure 5).

These unusual α–amino acids have important parts to play in the structures of some proteins.

Proteins

2. The α-amino acid building blocks



2.4 Chemical properties of α–amino acids

a. Acid/base nature

α-amino acids contain both acidic (-COOH) and basic (-NH2) groups. Unfortunately, though, the picture is not as simple as this. In the solid crystalline state the α–amino acids exist as zwitterions, formed by the transfer of protons (H+) from the -COOH to the -NH2 groups. For α–amino acids without acidic or basic side chains these zwitterions have charged groups but are neutral overall. This is shown on the left.

Zwitterions remain when the α–amino acid is dissolved in water at pH7. Addition of an acid, supplying more protons, produces ions with a surplus positive charge:

Addition of an alkali, providing hydroxide ions, produces ions with a surplus negative charge:

We can describe α–amino acids as amphoteric as they can react with both acid and alkali. They are effective buffers in biological systems.

The situation is more complicated in α–amino acids that have acidic or basic R groups , e.g. glu or lys (see Figure 3).

The structures in Figure 3 show the main ionic form of each α–amino acid at pH7.

At very low pH all α–amino acids exist as ions with an overall positive charge, while at high pH they exist as ions with an overall negative charge. For each α–amino acid there is a pH between these extremes at which its molecules are neutral overall. This value is called the isoelectric point for the α–amino acid. At its isoelectric point the α–amino acid molecules will not move when placed in an electric field. The separation technique called electrophoresis relies on molecules with different isoelectric points moving at different speeds when kept at a fixed pH and placed in an electric field (see Figure 6).

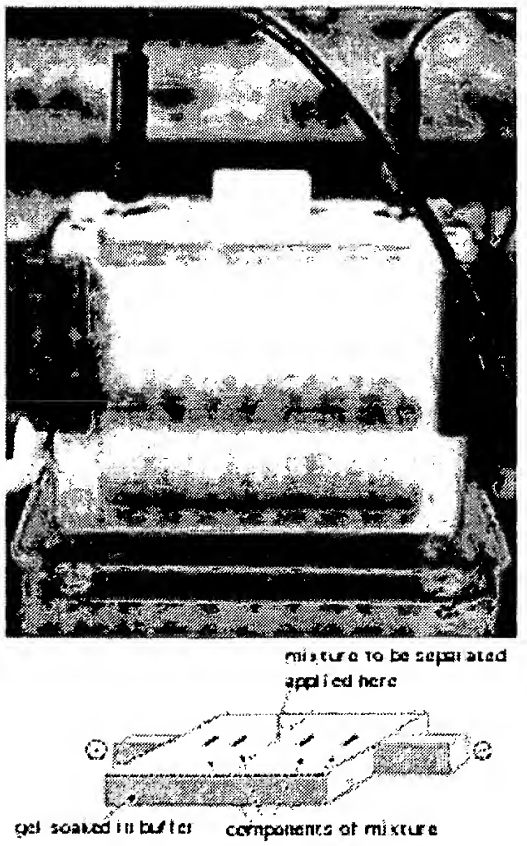


Figure 6
Electrophoresis. An electric potential difference is applied across a plate of gel. Molecules separate on the gel since they move at speeds that depend on their size and charge.

Questions:

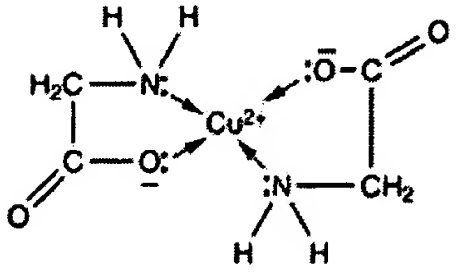
1. Why is glycine (M_r = 75) a solid at room temperature while ethanoic (M_r = 60) is a liquid?

2. Write balanced equations for the following, starting each equation with the ionic form given in Figure 3:
a. phenylalanine reacting with sodium hydroxide
b. methionine reacting with hydrochloric acid
c. aspartic acid reacting with excess hydrochloric acid
d. lysine reacting with excess sodium hydroxide.

3. Explain why the solubility of an α–amino acid in water is lowest at its isoelectric point.

Proteins

2. The α-amino acid building blocks



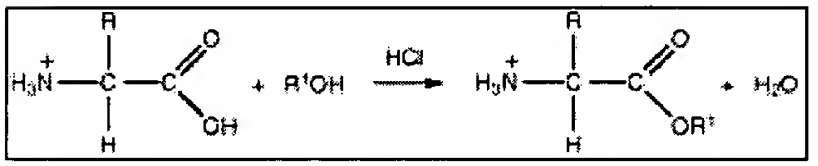
2.4 Chemical properties of α–amino acids
b. Other reactions of the α–amino and carboxylic acid groups
Complex formation

When α-amino acids form salts with d-block metals the α–amino and carboxylate groups form dative bonds to the metal ion. The effect is to wrap up (chelate) the metal ion with α–amino acid molecules. These salts often have characteristic colours - copper diglycinate (shown on the left) has a deep blue colour.

Questions (Group 2):

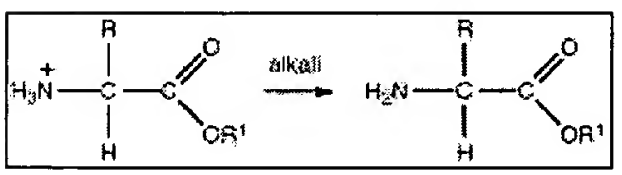
1. Copper diglycinate is a complex and the glycine molecules are the ligands . What type of ligands are the glycine molecules?

2. Why are glycine molecules able to act as ligands?



Condensation reactions

α–Amino acids form esters when heated with alcohols using dry hydrogen chloride as a catalyst . In these reactions the carboxylic acid group takes part in a condensatyion reaction , while the amino group forms a hydrochloride salt with the acid catalyst.

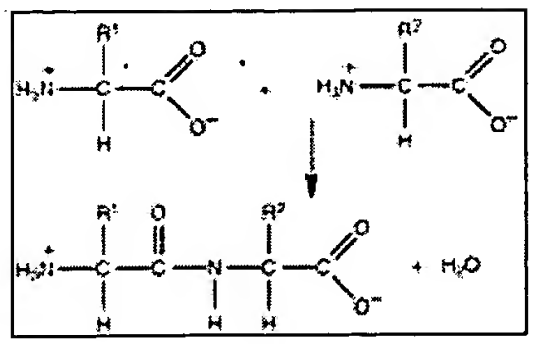


The H+ ions can then be removed by treatment with a suitable alkali.

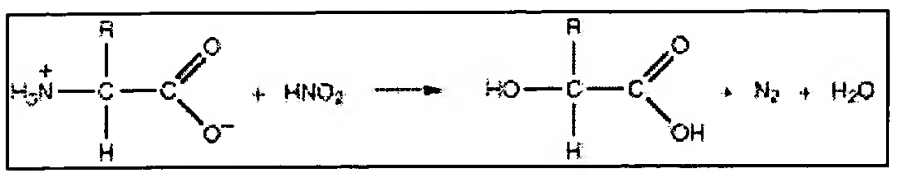
Question (Group 3):
Draw the structures of the esters formed from the following pairs of reactants:
a. alanine and butan-1-ol
b. aspartic acid and excess methanol

With the right conditions the amino group on one molecule and the carboxylic acid group on another will condense together to form a secondary amide :

This is the reaction that organisms use to build α–amino acid units into proteins. Why, then, is it so difficult for us to make



proteins synthetically using conventional organic techniques? In Chapter 4 you can read about the problems and the ways that scientists have solved them.



Reaction with nitric (III) acid

α–Amino acids react with nitric (III) acid (nitrous acid) at room temperature to produce hydroxy acids and nitrogen gas. One use of this reaction in determining the sequence of α–amino acids in a peptide is described in Investigation 4b. The amount in moles of amino groups in a sample can be measured by collecting and measuring the volume of nitrogen gas released.

Questions (Group 4):

1. Write out balanced equations showing how:
a. valine reacts with nitric (III) acid
b. lysine reacts with nitric (III) acid.

2. How can you make the nitric (III) acid needed in this reaction?

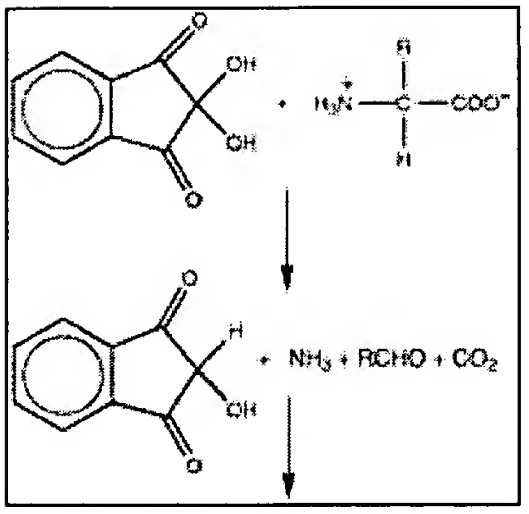
Proteins

2. The α-amino acid building blocks

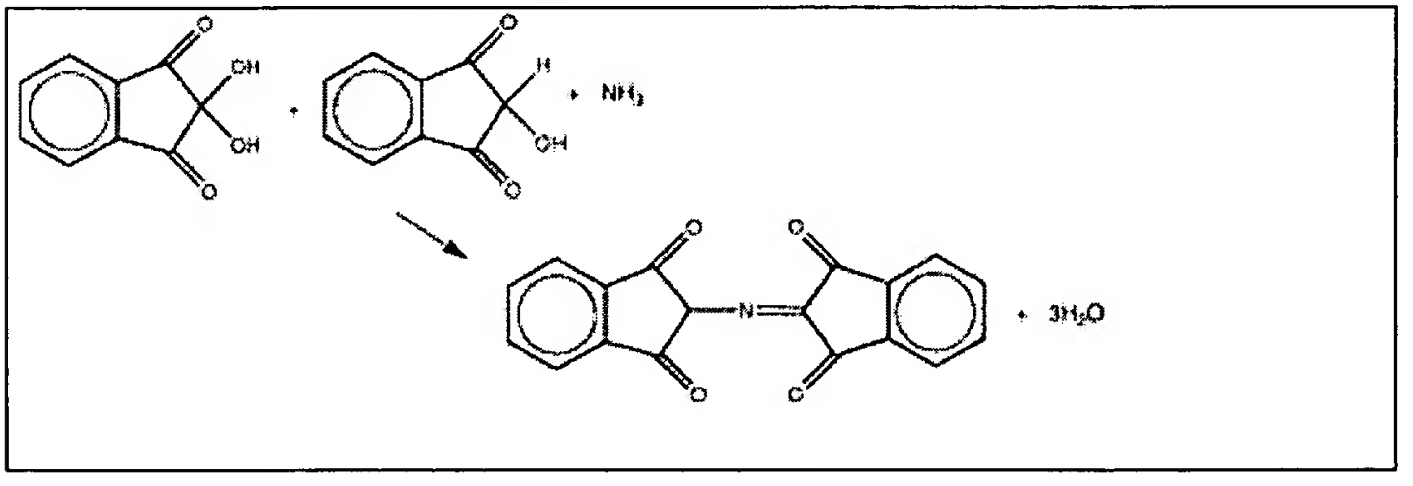
2.4 Chemical properties of α–amino acids
c. Some reactions of the R groups

The side chain group influences the chemical properties of an α-amino acid . A functional group in the side chain will add to the reactions that the α–amino acid can take part in.

Questions (Group 5):
Using the three letter abbreviations, list those α–amino acids with side chain groups that:
a. can react with an alcohol to form an ester
b. are unsaturated
c. would give a violet complex if a neutral solution of iron(III) chloride was added
d. would be hydrolysed in aqueous acid to give a -COOH group and ammonium ions
e. can react with an acid chloride to give an ester.



The ninhydrin reaction
Ninhydrin (or 1,2,3-indantrione monohydrate) is an important analytical reagent. It reacts with α–amino acids to produce a purple coloured compound. We can use this colour-forming reaction to locate the colourless α–amino acid spots on a chromatogram (see Part 2, Basic experiment 4b) or the intensity of the purple colour can be used as a way of measuring the amount of α–amino acid in a sample.
An outline scheme for the ninhydrin reaction is shown on the left and below.



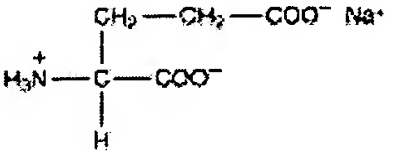
Reactions that can link R groups
In most proteins some α–amino acid units are covalently linked to other units at a distance along the same chain or in a different chain altogether. This happens when side chain R groups react together.

The most common type of R group link is the disulphide bridge (-S-S-) that forms from two cysteine R groups joining together. This type of cross-link is important in maintaining the three dimensional structure of protein molecules (see Figure 5 in chapter 2).

The making and breaking of a disulphide bridge are redox reactions .

Monosodium glutamate, MSG - a flavour enhancer

MSG is probably the most common additive that we use to increase and improve the flavour of food. It is the sodium salt of glutamic acid and has the `E number' E621.



The Chinese have been using it for a very long time as it is one of the components of soy sauce. The Chinese make soy sauce by the hydrolysis and fermentation of soya beans. Hydrolysis breaks down the soya proteins into amino acids and one of these is glutamic acid.

On its own MSG has only a faint meaty taste, but together with processed food products and other additives it has a powerful flavour enhancing effect. Food technologists use MSG in a variety of foods, mainly processed meats and dehydrated products.

Some people seem to react badly to MSG and experience the symptoms of what is called `Chinese restaurant syndrome'. These can include flushing, dizziness and palpitations. Although scientists do not think MSG is harmful it is no longer added to food prepared specially for babies and infants.

Fermented products containing soya.
Right - a `hot' red pepper paste.
Left - a savoury soy sauce.
Both are sources of monosodium glutamate an α-amino acid flavour enhancer.

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8. Investigating proteins

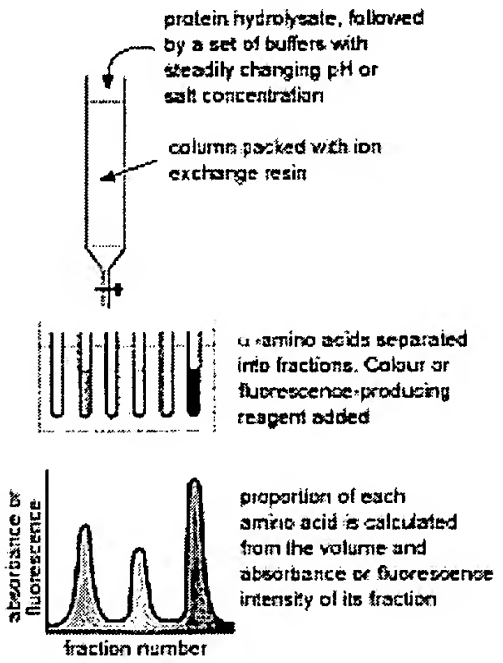


Figure 4
Ion exchange chromatography of amino acids.

8.2 Working out primary structure
Traditional methods

The first task here is to identify which α-amino acids make up the protein. Simple qualitative tests detect those α-amino acids that are present in high proportion (Basic experiment 4a, Investigation 4a). To carry out a full α-amino acid analysis, though, it is necessary to hydrolyse the protein completely then identify and measure the proportions of the different amino acids in the resulting mixture.

A common method for bringing about complete hydrolysis is prolonged heating under reflux with concentrated hydrochloric acid (Basic experiment 4b). The α-amino acids can then be separated using either HPLC (high performance liquid chromatography, or ion exchange chromatography (Figure 4).

After separation, the proportions of different α-amino acids can be measured by using reagents which give a colour or fluorescence with amino acids (Figure 4).

The a-amino acid analysis, together with the relative molecular mass of the protein, allows you to calculate the number of each α-amino acid unit in a molecule of the protein.

Questions:
α-Amino acid analysis of a peptide gave the following percentage composition by mass:

ala:gly:ser:val:cys = 30.3 : 15.3 : 14.3 : 31.9 : 8.2.

1. Calculate the mole ratio for the

α-amino acids present using the following relative molecular masses:

ala = 89, gly = 75, ser = 105, val = 117, cys = 121.

2. The relative molecular mass of the peptide is 1469. Calculate the number of each α-amino acid unit in each molecule of the peptide.

The arrangement of a-amino acids in sequence can be investigated using chemical techniques. The α-amino acid group at either the free α NH_3^+ or α COOα end of the peptide can be labelled by joining new chemical groups to them (Investigation 4b). Chromatography can identify these terminal α-amino acids after peptide hydrolysis (Figure 5). This hardly scratches the surface of the problem, though, for a large polypeptide molecule.

One way to dig deeper into the primary structure is to use the Edman degradation. This allows you to label the α-amino acid at the free α NH_3^+ end

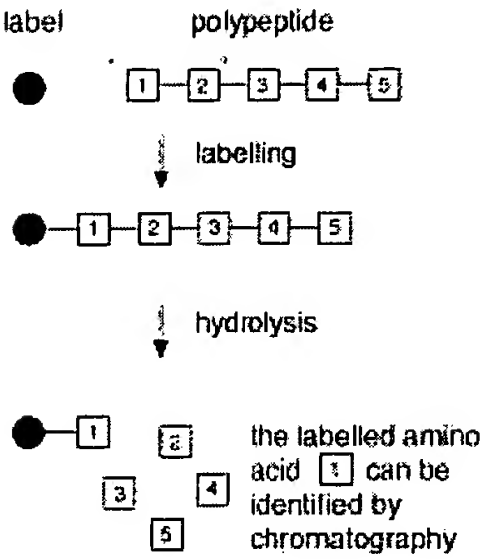
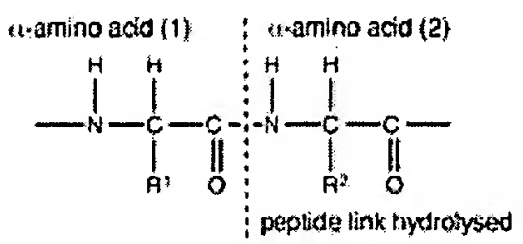


Figure 5

-$NH3^+$ or -COO^- terminal analysis. The α-amino acids are numbered from the labelled end of the polypeptide chain.

and selectively release it without hydrolysing the rest of the peptide. You can then identify the labelled amino acid and repeat the labelling and releasing steps on the remaining chain, gradually working out the sequence. However, as more and more α-amino acids are removed less and less material remains, so it is not always possible to sequence an entire protein molecule using just this technique.



Scientists have developed automatic machines called ' sequenators', based on this principle, that can sequence a chain of up to approximately fifty residues. This takes several days but needs the minimum of supervision. The sequenator holds the shortening peptide chain, **immobilised** on a film, while the reagents and solvents for separating the products are passed over. The machine then uses HPLC to identify the labelled α-amino acids as they wash through in sequence.

To sequence larger peptides and proteins it is necessary to use reagents or enzymes that bring about the hydrolysis of specific peptide links :

reagent or enzyme	**peptide links broken**
cyanogen bromide (CNBr)	α-amino acid 1 = met
trypsin	α-amino acid 1 = lys or arg
chymotrypsin	α-amino acid 1 = phe, trp, tyr

Table 1

Table 1 (on the left) shows what α-amino acid (1) must be for each of three reagents or enzymes to bring about the hydrolysis of the peptide link.

A large polypeptide chain must be first broken into fragments that are small enough to be sequenced. You can only work out how to fit the fragments together in order, though, if you have more than one set of fragments from hydrolysing the protein using different methods. This gives fragments with overlapping sequences.

The example below shows how this works:

fragments from trypsin hydrolysis	**fragments from chymotrypsin hydrolysis**
gly-gly-ala-trp-val-lys	ser-glu-phe
leu-ala	arg-gly-gly-ala-trp
ser-glu-phe-arg	val-lys-leu-ala

trypsin fragments	ser-glu-phe-arg gly-gly-ala-trp-val-lys	leu-ala
peptide sequence:	ser-glu-phe-arg-gly-gly-ala-trp-val-lys-	

leu-ala

chymotrypsin fragments: arg-gly-gly-ala-trp
ser-glu-phe val-lys-leu-ala

Proteins with sub-units must have their polypeptide chains separated before each is sequenced.

Proteins may have more than one polypeptide chain linked by disulphide bridges (-S-S-). These bridges can be located by hydrolysing the peptide, oxidising the -S-S- bonds, and sequencing the fragments.

Question:
Use the following data to work out the sequence of a polypeptide that has 13 α-amino acid residues.

fragments from trypsin hydrolysis
ser-tyr-ser-met-glu-his-phe-arg
trp-gly-lys
gly-ala

fragments from CNBr treatment
ser-tyr-ser-met
glu-his-phe-arg-trp-gly-lys-gly-ala

fragments from chymotrypsin hydrolysis
ser-tyr
ser-met-glu-his-phe
arg-trp
gly-lys-gly-ala

Proteins

4. Protein synthesis and protein engineering

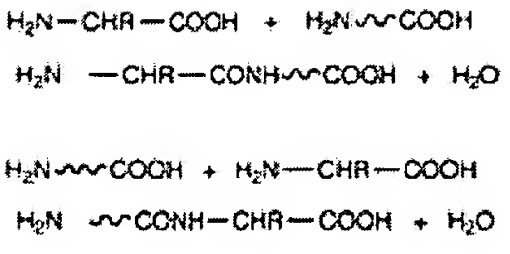
If we are to make long polypeptide chains with α-acid monomers in a specified sequence then we must have a very tight control of the reactions we use. In this chapter you will see the complex chemical systems that organisms use and how scientists use the techniques of protein engineering

to tailor modifications. But first we will look at the more conventional, though elegant, approach of the synthetic organic chemist.

4.1 Solid phase peptide synthesis

The synthetic organic chemist faces several problems:

1. The carboxylic acid group (-COOH) in an α-amino acid is not reactive enough to give a condensation reaction with the amine group (- NH2) of another amino acid using mild conditions.
2. As the polypeptide chain is built up unit by unit, care must be taken not to expose it to conditions that would hydrolyse the peptide links already made.
3. Many α-acids have side chains with functional groups that may react under the conditions used to make the peptide links.



4. A polypeptide chain has two reactive ends; the free -NH2 and the free -COOH. The synthetic chemist must make sure that each extra α -amino acid unit adds on to the correct end of the polypeptide chain being made (see figure on the right).
5. The chain must be elongated by one α -amino acid unit at a time. This means one linking reaction for each α -amino acid in the chain, followed by separation of the products. Each reaction must, therefore, have a high yield and each purification step must separate most of the product.

Chemists solve the problems above as follows. Problems 1 and 2 by using a coupling reagent that increases the reactivity of the - COOH group under mild conditions.

Problem 3 by protecting reactive side chains with blocking groups that they can remove under mild conditions when all the links are complete.

Problem 4 by blocking the α -amino acid and polypeptide groups that they do not want to join, again using groups they can remove easily later.

Problem 5 by attaching the growing polypeptide chain to an insoluble support made from a resin. They then wash away soluble by-products and unused reagents before adding the next α -amino acid to the chain. When the synthesis is complete the polypeptide is detached from the resin.

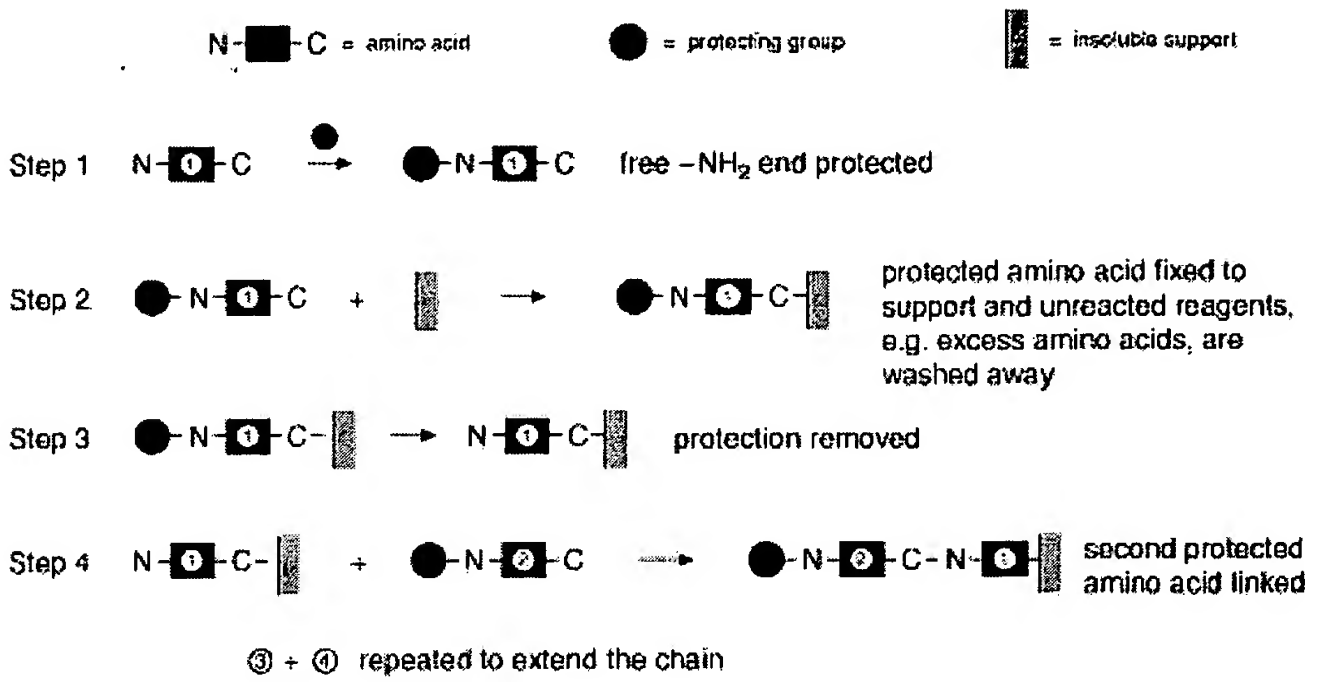


Figure 1a
Solid state peptide synthesis.

The 1984 Nobel Prize for Chemistry was awarded to Professor Merrifield of Rockefeller University for developing an automated version of this technique capable of producing the hormone insulin (51 α -amino acids). Figure 1a summarises the sequence of steps involved. Figure 1b gives the primary structure of the two chains in human insulin. Figure 2 on page 1 shows the molecule in 3D.

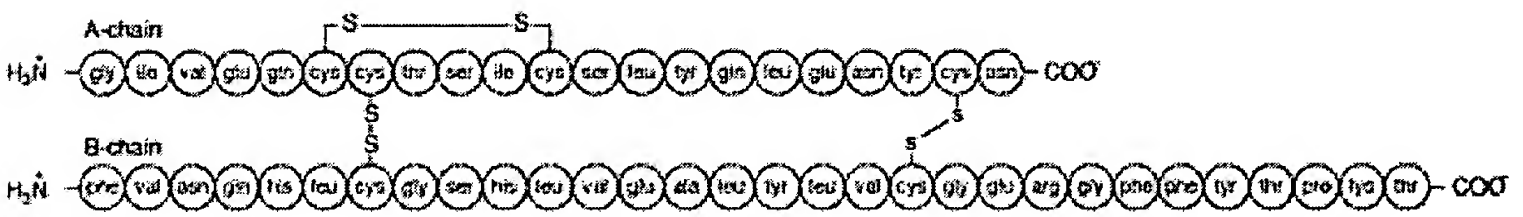


Figure 1b
The primary structure of the two polypeptide chains in human insulin.

Question:

How many different tripeptides (three α -amino acids linked together) could you make using three different α -amino acids?

Proteins

3. Protein structure

α-amino acids

Proteins, whether large or small, are remarkably intricate pieces of molecular engineering. To understand what they are like and how they work, we need to be able to imagine what their molecules might look like. In this chapter you will see how scientists picture protein molecules and you will be introduced to some of the technical terms they use to describe them.

Peptides are molecules made by joining α-amino acid units. Peptides with more than about 50 α-amino acid units are called **polypeptides**. **Proteins** are made from a single polypeptide molecule or from several linked together. The links can be covalent bonds, using disulphide bridges (-S-S-), or non-covalent, intermolecular forces (see Figure 1).

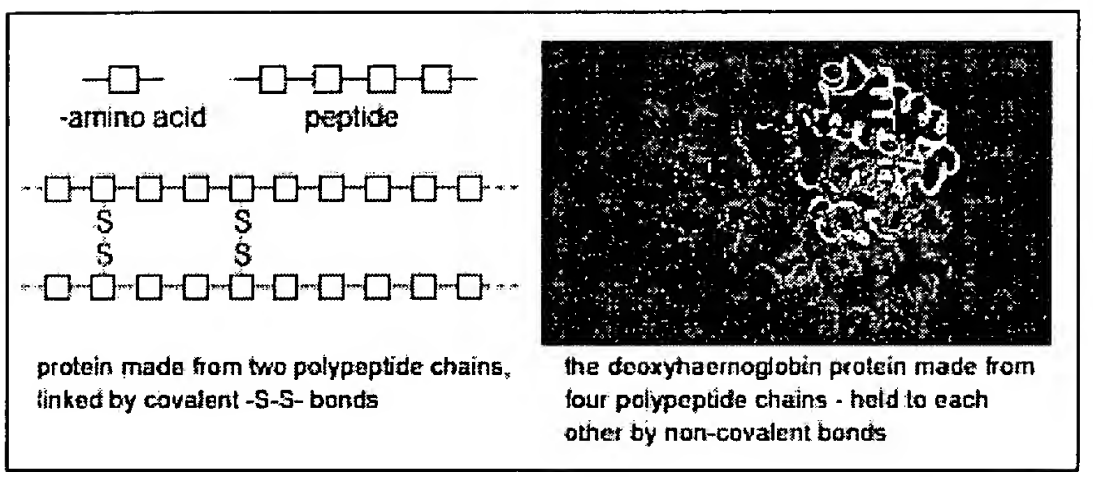


Figure 1
Peptide and protein structure

3.1 Primary structure

All polypeptide molecules (**chains**) share a common structure called the **peptide backbone**.

The backbone differs in length from one polypeptide to another. In nature all polypeptide chains are **linear**, not branched.

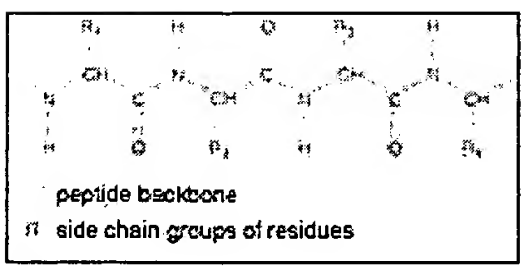


Figure 2
A small section of a polypeptide chain

In addition there are **side chain groups (residues)** that belong to each α–amino acid unit (Figures 2, 3 on this page, and Figure 3 Chapter 2). The number of each type of residue and their order along the backbone give each polypeptide its unique properties.

The **α-amino acid composition** of a polypeptide tells us the numbers of each α-amino acid unit in the molecule. The **primary structure** of a polypeptide tells us the sequence in which the different units are linked.

Protein chemists write amino acid sequences with the free -NH2 (or $-NH3^+$) end of the molecule on the left and the free -COOH (or -COO-) end on the right.

In Chapter 4 you will see how genes determine the primary structures of proteins.

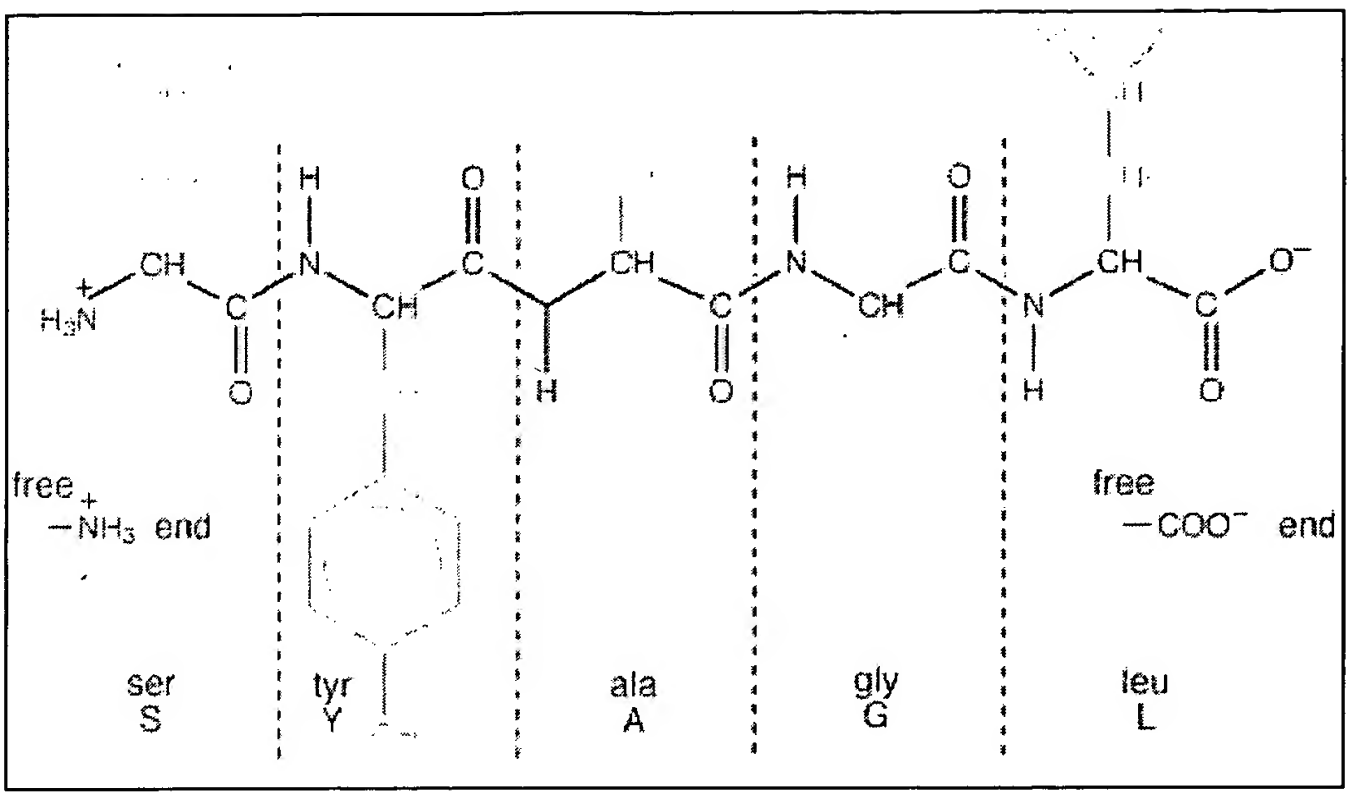


Figure 3
Primary sequence of a small peptide

Questions:

1. Refer to Figure 3 on page 4 and describe the sequence of the following section of a primary structure of a peptide using:
a) the three letter α-amino acid codes
b) the one letter codes
c) the skeletal formula.

2. Draw the full structure of ser-gly-ala.

Proteins

3. Protein structure

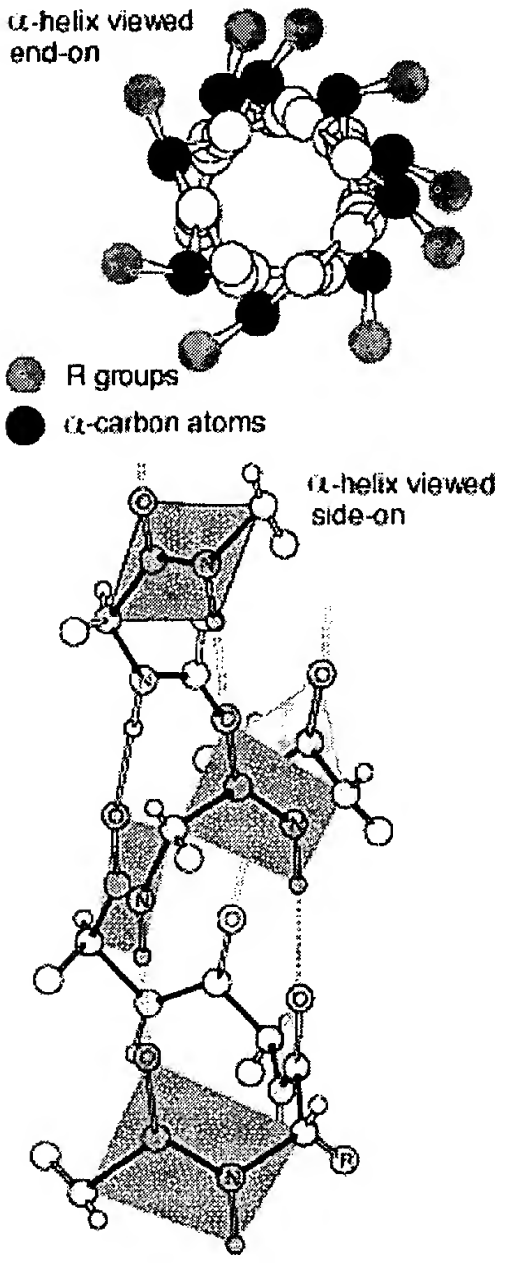


Figure 8
The α-helix.

3.2 Secondary structure (continued)

We can describe the arrangement of atoms around the peptide link (the conformation) by giving the degree and direction in which the Cα–CO and N-Ca bonds are rotated. When a number of successive peptide links have identical rotations the polypeptide chain takes up a particular secondary structure.

There are several types of secondary structure, but we will concentrate on just two: the α-helix and the β-pleated sheet. In both cases you will see how the regular conformation allows the structure to be stabilised by forming many relatively strong hydrogen bonds .

The α-helix

The α-helix is like a narrow-bore tube. The polypeptide backbone is coiled up like a very tight clockwise screw thread or the cord of a telephone. The peptide link plates form the wall of the tube with the Cα atoms projecting a little from the surface. The side chain groups, attached to the Cα atoms, project outwards from the wall of the tube (figure 8).

As you follow the helix around through 36 α-amino acid units you make 10 complete 360Υ turns and travel 5.4 nm in the forward direction (1 nm = $1x10^{-9}$m).

The α-helix conformation has a particular stability for two main reasons. Firstly the side chain groups are quite well separated. Secondly, and most importantly, each peptide link is involved in two hydrogen bonds. The C=O is hydrogen bonded to the N_H of the peptide link four units ahead in the primary structure , while it follows that the N_H is hydrogen bonded to the C=O of the peptide link four units behind (figure 7). The atoms involved are arranged linearly (figure 8) so that the hydrogen bonds are nearly at their maximum strength. The hydrogen bonds run down the length of the a-helix tube and lock the conformation in place.

Questions:

1 How far does the α-helix extend for each complete turn (this is called the 'pitch' of the helix)?
2 How far does the α-helix extend for each a-amino acid unit?
3 By how many degrees does the helix twist for each α-amino acid unit?
4 How many α-amino acid units does it take to make one complete turn?

2. Draw the full structure of ser-gly-ala.

The β-pleated sheet

In the β-pleated sheet structures (Figure 9) the polypeptide backbone is nearly

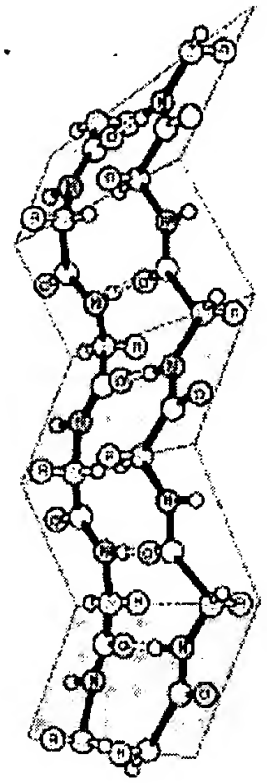


Figure 9a
3D view of antiparallel β-pleated sheets

fully stretched. This allows the peptide N_H and C=O to point out at right angles to the line of the backbone. N_H and C=O groups alternate along each edge. When two or more of these extended chains (called β-**strands**) are side by side hydrogen bonds form between them to give an almost two dimensional sheet that is pleated like a skirt or the bellows of an accordion.

Alternate β-strands can run in the same direction to give a **parallel** β-pleated sheet or in opposite directions to give an **antiparallel** β-pleated sheet.

The pleating in each case allows for the best alignment of the hydrogen bonded groups.

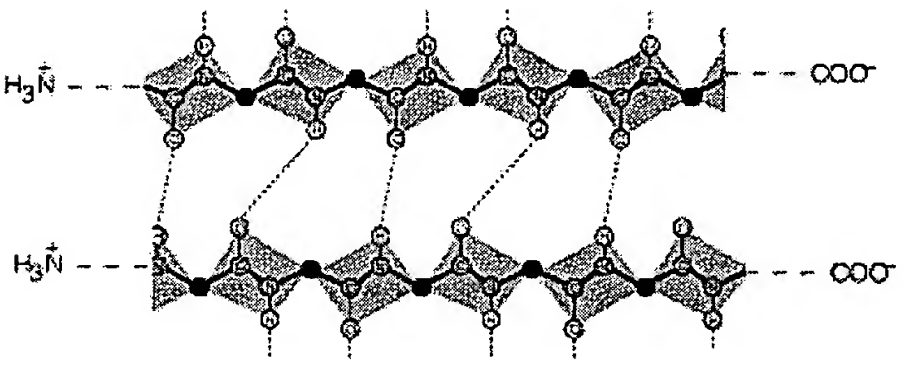


Figure 9b
Parallel β-pleated sheets

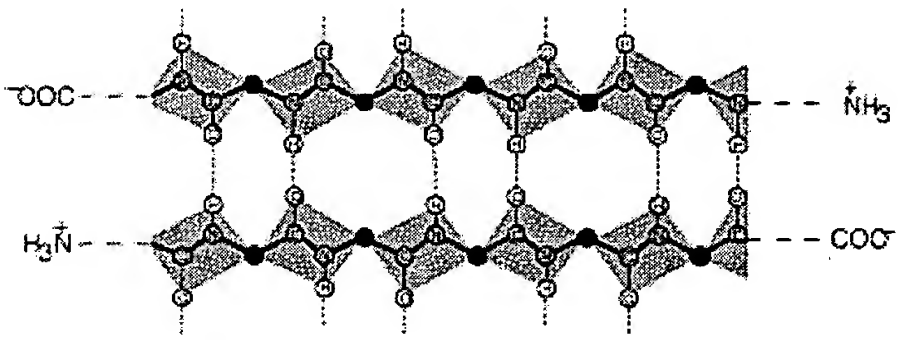


Figure 9c
Antiparallel β-pleated sheets

Proteins

1. Focus on proteins

Figure 1.
Products containing proteins

1.1 Why are proteins important to us

Proteins make up about 15% of the mass of the average person. Protein molecules are essential to us in an enormous variety of different ways. Much of the fabric of our body is constructed from protein molecules. Muscle, cartilage, ligaments, skin and hair - these are all mainly protein materials.

In addition to these large scale structures that hold us together, smaller protein molecules play a vital role in keeping our body working properly. Haemoglobin, hormones (such as insulin, shown in Figure 2), antibodies , and enzymes are all examples of these less obvious proteins.

Whether you are a vegetarian or a ' meat eater' you must have protein in your diet. The protein in the food we eat is our main source of the chemical building blocks we need to build our own protein molecules.

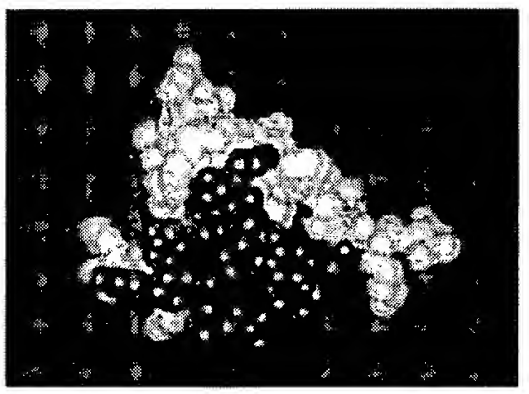


Figure 2 *Computer generated image of the protein insulin. The molecule consists of two chains called the A-chain and the B-chain, coloured red and white in this picture.*

1.2 What are proteins?

Proteins belong to a class of organic compound called **polyamides**. Polyamides are polymers where the monomer units are held together by **amide groups**. The monomer units in proteins are called **α-amino acids**. The amide group -CO-NH- joining two α-amino acids is often called a **peptide link**, so scientists sometimes call single polymer chains made from α-amino acids **polypeptides**. Proteins can be made from a single polypeptide chain or from several polypeptide chains joined together.

Only twenty α-amino acids are commonly found in proteins; so how can they form such a wide variety of polymers with such different properties and functions? The simplest way to appreciate this is to imagine that the α-amino acids are like letters in the alphabet and that proteins are like words. Nature makes many different proteins from twenty α-amino acids, in the same way that we can make a dictionary full of words from just twenty six letters. But there is an important difference. Proteins contain many more α-amino acids than words contain letters: some protein chains contain several hundred α-amino acid units. The scope for variety is immense.

1.3 Proteins in foods

Some micro-organisms in the soil can **fix** molecular nitrogen from the air into water-soluble ions such as nitrate ($NO3^-$) and ammonium (NH_4^+). Plants can then absorb this inorganic nitrogen and use it to make their proteins and other nitrogen containing molecules. Animals feed off the plants and recycle the α-amino acid building blocks into their own protein. Humans can use both plants and animals as a source of dietary protein. Excretion, death and decay ensure that the nitrogen compounds produced can be re-used by other organisms. This **nitrogen cycle** is a well known example of how the elements of life are used

over and over again.

Meat makes the largest contribution to the protein in the diet of the average westerner, with milk and bread playing smaller parts. Cereals make a much larger contribution in the poorer regions of the world. See Practical Work, Section 1.

Question:
Why is meat an expensive source of protein, compared with other protein containing foods?